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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
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HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361

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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

PC 059102

SUBJECT: PP#0F2423 and FAP#0H5277 Chlorpyrifos-methyl on stored grains. Amendment of 9/14/82.

FROM: R. B. Perfetti, Ph.D., Chemist
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TO: Jay Ellenberger
Product Manager (12)
IRB, RD (TS-767)
and
Toxicology Branch
Hazard Evaluation Division (TS-769)

THRU: Charles L. Trichilo, Chief
Residue Chemistry Branch
Hazard Evaluation Division (TS-769)

This amendment is in response to our memo of 3/13/81 in which several deficiencies in the subject petition were outlined. These deficiencies are listed below in the order in which they appeared in the original memo. The petitioner's responses to the deficiencies follow this list.

Deficiencies:

1a. A metabolism/degradation study on a stored grain is needed. Special attention should be given to the amount and nature of any conjugates of metabolites of chlorpyrifos-methyl formed during storage. Such studies have been required previously for temporary tolerance proposals on stored commodities and extensive alteration of the terminal residue in these commodities has been observed.

1b. Additional metabolism studies on a ruminant (preferably a lactating goat) and chickens are needed. These studies should involve feeding of ^{14}C-labeled chlorpyrifos-methyl

and identification and quantitation of the terminal radioactive residue in meat, milk, poultry and eggs. In all the animal metabolism studies submitted to date, no attempt to identify the nature of the radioactive residue in tissues was made even though radioactive residues in sheep fed labeled chlorpyrifos-methyl ranged up to 11.8 ppm in fat and were also significant in other tissues. No metabolism studies on a lactating ruminant or chickens (eggs) were performed.

2a. Adequate analytical methods for determination of chlorpyrifos-methyl and free 3,5,6-trichloro-2-pyridinol in grains, processing fractions, meat, milk, poultry and eggs are available. This conclusion, however, is tentative until both the questions regarding the metabolism in grains and animals discussed above are resolved and a successful method trial is completed. The method trial of the appropriate procedures will be initiated at such time as all of the other deficiencies in the petition are resolved. Additional methods or modifications of present methods which will allow determination of conjugates or metabolites of chlorpyrifos-methyl in various r.a.c.'s may be needed. Also, confirmatory procedures (different glc columns or TLC, etc.) will be needed prior to establishment of tolerances.

2b. In the residue data submitted, identical samples analyzed via the method which determines parent vs determination using the method which measures parent and 3,5,6-trichloro-2-pyridinol as the alcohol always showed much lower residue values when the latter method was used. This question should be addressed. This discrepancy may be due to incompleted hydrolysis of chlorpyrifos-methyl in the grain samples.

3a. The proposed 6 ppm tolerance level for stored grains is appropriate since this is the maximum application rate and combined residues of parent or any metabolites which would occur upon storage could not exceed this value. We consider the high residue value for rice aberrant. This conclusion does not negate, however, our concerns over the nature of the residue discussed above. We will need to know whether conjugates of the pyridinol or perhaps the des-methyl or other compounds would be released from grains containing "aged" residues of chlorpyrifos-methyl when the alkaline hydrolysis described in Method ACR 78.19 is employed. Also, as mentioned above, an explanation of the difference in residue levels found in identical samples of grain analyzed via methods ARC 78.18 and ARC 78.19 should be submitted.

3b. The proposed food-additive tolerances, 160 ppm for refined corn oil and 30 ppm for rice milling fractions, are appropriate. Based on the processing/milling studies submitted, appropriate tolerance levels for other grain byproducts are:

Sorghum milling fractions (except flour)	90 ppm
Barley milling fractions (except flour)	90 ppm
Corn soapstock	40 ppm
Oats milling fractions (except flour)	130 ppm
Wheat milling fractions (except flour)	30 ppm

As discussed above, these recommendations are tentative pending satisfactory resolution of questions regarding the nature of the teminal residue in grains and by-products and the adequacy of the proposed methods for determining metabolites and/or conjugates of these compounds which are of concern. Pending the results of the additional studies required, further processing/milling experiments may be needed.

4a. The method used to determine 3,5,6-trichloro-2-pyridinol and chlorpyrifos-methyl both as the alcohol in swine, calves, cows and poultry used a methanol extraction but no hydrolysis step and thus, in all probability, did not determine conjugates, if any, of metabolites of chlorpyrifos-methyl. Our concern over the possibility of the formation of conjugates of the pyridinol and other possible metabolites of chlorpyrifos-methyl in animals must be resolved by the additional metabolism studies required above. Depending on the outcome of these metabolism studies, additional feeding studies in livestock employing a hydrolysis step in the method of analysis may be needed.

4b. All of the grains involved in this petition are major feed items which can comprise up to 80 and 70% of the diets of cattle and poultry respectively. Based on the feeding studies submitted, this chemical is classed in category 2 of Section 180.6(a) with respect to meat, milk, poultry and eggs and therefore tolerances are needed for these commodities. No estimation of appropriate tolerance levels for these r.a.c.'s can be made, however, until such time as the deficiencies described in 4a above are resolved.

5. The International Tolerance Sheet is attached. The proposed Codex tolerances for milk (0.01 ppm), maize (10 ppm), rice (0.1 ppm) and sorghum (10 ppm) are expressed in terms of parent compound only. Therefore, these tolerances will not be compatible with U.S. tolerance regulations which will include parent plus at the very least, 3,5,6-trichloro-2-pyridinol and perhaps other

metabolites. It is also doubtful that the Codex tolerance levels will be compatible with the final U.S. levels.

At this time, we foresee no mechanism by which the U.S. tolerances could be made compatible with the Codex proposals other than via extensive modification of the Codex tolerances.

Response to 1a and 1b:

The petitioner has submitted a study employing radiolabeled chlorpyrifos-methyl applied to stored corn and wheat grain as well as metabolism studies on lactating goats and laying hens. These are discussed in detail below.

The stored grain experiment involved treating corn and wheat with ^{14}C-labeled chlorpyrifos-methyl at a rate of 32.4 ppm. The samples were then stored at 25°C and collected for analysis at 0, 30, 90 and 180 days post-treatment. Some samples were also designated for analysis after 360 days of storage.

The 30, 90 and 180 day samples were extracted with acetone/water and centrifuged. The supernatant was assayed for radioactivity and then concentrated for hplc and TLC analysis. The insoluble residues after acetone/water extraction were dried and saved for combustion analysis and hydrolysis. The residue was hydrolyzed with NaOH in 80:20 methanol:water on a steam bath for 2 hours except for the 180 day insoluble residue sample which was hydrolyzed twice with base. All of the samples were then cooled, centrifuged and aliquots of the supernatants were assayed for radioactivity. The supernatants were then acidified and partitioned with ether. The ether extracts were taken to dryness and then redissolved in methanol for hplc and TLC analysis. The insoluble residues were saved for combustion analysis.

Acetone/water extractable residues decreased with time to a low value of ca. 67% of the total radioactivity. There was a corresponding increase in unextractable radioactivity in the grain. After base hydrolysis, 4.9 to 19.1% of the radioactivity remaining in the grain was relaeased with 0.7 to 8.5% of the total radioactivity remaining insoluble. In all, 81.2 to 97.5% of the total radioactivity was extracted from treated wheat or corn grain. Unextractable or aqueous soluble radioactivity ranged from 0.8 to 9.2%. Acetone extracts of wheat or corn showed decreasing amounts of parent with time (ca. 20 to 40% of total radioactivity after 180 days storage) with corresponding increase in trichloropyrindinol (12.7 to 29.1% after 180 days), the mono-acid (14.1 to 20.4% after 180 days) and the S-methyl isomer (a maximum of 0.6% after 90 days storage).

After base hydrolysis, recovered radioactivity was made up of 3.3 to 12.4% of combined trichloropyridinol and the S-methyl isomer (percentages of initial radioactivity) and 0.1 to 8.4% mono-acid. It is our judgement that the nature of the residue in stored grains is adequately understood. The terminal residue will consist of parent, 3,5,6-trichloro-2-pyridinol, the sodium mono-acid of chlorpyrifos-methyl and the S-methyl isomer of chlorpyrifos-methyl in decreasing order of abundance. Even though the present method determines parent, mono-acid and 3,5,6-trichloropyridinol in grains we defer to TOX the question of whether they are concerned over residues of the mono-acid at a maximum level of 1.5 ppm and the S-methyl isomer of chlorpyrifos-methyl at a maximum level of 0.05 ppm in grains. Residues of these compounds in grain milling fractions and corn oil may be proportionately higher. If TOX expresses concern over these compounds additional methodology (for the S-methyl isomer) including validation data and blank crop values as well as possible processing and feeding studies may be needed. The petitioner should be so informed.

The metabolism study in laying hens reflected dosing from chickens with ^{14}C-labeled chlorpyrifos-methyl for up to 10 consecutive days at a level equivalent to 25 ppm in the diet. Eggs were collected daily and separated into whites and yolks. Excreta was also sampled daily. Within 16 hours of the last dose the animals were slaughtered and liver, kidneys, heart, muscle, skin and fat samples were collected.

The samples were assayed for radioactivity by oxidative combustion followed by liquid scintillation counting.

Total radioactivity in egg whites and yolks appeared to plateau after ca. 3 days. Radioactive residues ranged from <0.01 to 0.03 ppm in egg whites and from <0.01 to 0.096 ppm in yolks during the experiment. Radioactivity in various tissues ranged from <0.01 to 0.348 ppm (fat) during the study. The major portion of the radioactive residue (up to ~77%) was observed in the excreta.

Poultry tissues and eggs were extracted with acetonitrile; partitioned with hexane and the acetonitrile was stripped in vacuo. The residue was then dissolved in methanol, radioassayed and analyzed via TLC and hplc. Excreta samples were extracted with methanol and the residue was then filtered and the sample taken to dryness and resuspended in methanol for radioassay and analysis again via TLC and hplc.

The tissue and egg material remaining after extraction was hydrolyzed with NaOH in methanol/water (80:20) under reflux for 2 hours. After cooling the samples were centrifuged, acidified and the methanol was removed in vacuo. The aqueous

solution was extracted with ether, the ether extracts were dried and the samples were taken to dryness. The residue was redissolved in methanol for radio assay and identification as above.

Samples of the kidney, fat and egg yolks were pooled and used for identification; >75% of the radioactivity in any sample was extracted into acetonitrile and another 12.6 to 18.5% of the radioactivity could be extracted into ether after base hydrolysis. Essentially all of the radioactive residue in the egg or tissue could be extracted during sample workup. Methanol extracts of excreta also contained >90% of the radioactivity observed. Poultry kidney contained 67.1% of the chloropyridinol and 22.6% of the mono-acid. The fat samples showed 74.8% of parent compound, 0.4% of the S-methyl isomer, 1.1% of the trichloropyridinol and 2.3% of the mono-acid. Parent, trichloropyridinol and mono-acid at ~16%, ~20%, and up to 26.7% respectively were observed in egg yolks. All of the above were based on TLC analysis. The same general values were observed when the samples were analyzed *via* hplc. Trichloropyridinol comprised the major portion of the radioactive residue (>60%) in excreta with smaller amounts of parent, mono-acid and S-methyl isomer also being observed. We conclude that the nature of the residue in poultry is adequately understood. The terminal residue will consist of chlorpyrifos-methyl, 3,5,6-trichloro-2-pyridinol and the mono-acid and S-methyl isomer of chlorpyrifos-methyl. Since, however, present methods for poultry and eggs do not involve a hydrolysis step to convert the mono-acid to the pyridinol and therefore determine only parent compound and the trichloropyridinol we defer to TOX the question of whether they would be concerned over residues of both the mono-acid of chlorpyrifos-methyl at a maximum value of 0.13 ppm and residues of the S-methyl isomer at a maximum value of 0.01 ppm in poultry and eggs. If TOX expresses concern over these compounds, additional methodology including validation data, blank values and sample chromatograms as well as possible feeding studies will be required. The petitioner should be so informed.

The lactating goat metabolism study involved treated two animals with ^{14}C-labeled chlorpyrifos-methyl at a level of *ca.* 30 ppm for 7 consecutive days. Medication was performed twice daily *via* gelatin capsules. Milk was collected twice a day. Blood, urine and feces were collected daily. Expired CO_2 was collected from one of the goats on day 4 during a 10 hour period. Approximately 14 hours after the last dose the animals were slaughtered and blood, liver, kidney, heart, muscle and fat samples were taken. All CO_2, milk, blood, urine, feces and tissue samples were radioassayed. Radioactivity in milk appeared to plateau after *ca.* 36 hours with residues ranging from 0.013 to 0.0314 ppm. Residues of

radioactivity in milk showed a preference for the fat where the range observed was 0.0365 to 0.1423 ppm vs 0.0082 to 0.018 ppm in skim milk. Radioactivity in blood plateaued rapidly after dosing. A major portion of the radioactive dose (>85%) was excreted in the urine with an additional 1.5% being excreted via the feces. No significant radioactivity (<0.1% of a single dose) was observed in expired air. Radioactive residues in tissues ranged from 0.011 ppm to 0.615 ppm (kidney). Significant radioactivity was also observed in the blood, rumen and the large and small intestine.

Tissues and milk fat were extracted with acetonitrile, filtered, partitioned with hexane and the acetonitrile was stripped in vacuo. The residue was redissolved in methanol for radioassay and TLC and hplc analysis. The remaining filter cakes were hydrolyzed with base as discussed above for poultry and eggs. The final residue was also taken up in methanol for radioassay and identification via TLC and hplc. Urine samples were analyzed directly via TLC and hplc followed by hydrolysis using a mixture of beta-glucuronidase and sulfatase. The samples were also analyzed using TLC and hplc.

Liver, kidney, heart, fat, blood and milkfat samples allowed >70% of the total radioactivity to be extracted into acetonitrile with 1.6 to 23.4% of the total radioactivity residue remaining insoluble in acetonitrile. Base hydrolysis allowed another 5.7 to 15.4% of the radioactivity in liver, kidney, heart or blood samples to be extracted into ether. In all, >80% of the radioactive residue in all samples was extractable either before or after hydrolysis. After TLC analysis, the major portion of the radioactivity in liver, kidney and heart was 3,5,6-trichloro-2-pyridinol (66.4 to 75.1%) with smaller amounts of parent (non-detectable to 2.9%), Smethyl isomer (non-detectable to 0.5%) and mono-acid (1.9 to 8.7%) also being observed in these tissues. Parent compound comprised the major portion of the radioactive residue in fat and milkfat with the trichloropyridinol, the mono-acid and the S-methyl isomer also being observed in decreasing order of abundance. In blood, 3,5,6-trichloro-2-pyridinol represented 68.6 to 73.1% of the total radioactivity with smaller amounts of the remaining three compounds also found to be present. Results from the hplc analysis were analagous to the TLC results. Only 3,5,6-trichloro-2-pyridinol was observed in liver, kidney or blood after base hydrolysis and TLC analysis. Analysis utilizing hplc of the base hydrolyzed samples indicated that parent and the three metabolites were all present to some extent. In urine, the 3,5,6-trichloro-2-pyridinol comprised the major portion of the radioactive residue with or without enzyme hydrolysis. The next most significant amount of radioactive residue represents the mono-acid with much smaller amounts of parent and S-methyl isomer being observed.

The nature of the residue in ruminants is adequately understood. The terminal residue will consist of chlorpyrifos-methyl, 3,5,6-trichloro-2-pyridinol and the S-methyl isomer and mono-acid of chloropyrifos-methyl. Since the present methods of enforcement do not involve a hydrolysis step to convert the mono-acid to the pyridinol and therefore measure only parent and 3,5,6-trichloro-2-pyridinol we defer to TOX the question of whether they would be concerned over residues of both the S-methyl isomer of chlorpyrifos-methyl at a maximum level of 0.01 ppm and of the mono-acid at a maximum level of 0.05 ppm in meat and at maximum levels of 0.001 and 0.01 ppm respectively in milk. If TOX expresses concern over these compounds additional methodology including validation data, blank values and sample chromatograms as well as possible feeding studies will be needed. The petitioner should be so informed.

We have just been informed by TOX in an oral communication (R. Landolt, 1/17/83) that they would not be concerned over residues of the mono-acid or S-methyl isomer of chlorpyrifos-methyl in stored grains, meat, milk, poultry or eggs even at the maximum levels discussed above. Therefore we consider the nature of the residue in stored grains and animals to be adequately understood. The terminal residue of concern will consist of chlorpyrifos-methyl and 3,5,6-trichloro-2-pyridinol.

Response to 2a:

Based on the metabolism studies discussed above, it is our judgement that adequate analytical methods are available for enforcement purposes pending, of course, completion of a successful method trial. We are initiating such a trial at this time. The main procedure for determination of chlorpyrifos-methyl and 3,5,6-trichloro-2-pyridinol in grains is Method 78.19 with 78.18 and 80.7 being acceptable for confirmatory purposes. With respect to meat, milk, poultry and eggs the primary procedures are Methods 77.6 for parent and 78.9 for the pyridinol. TLC methods described in the metabolism studies as well as other methods submitted originally in this petition would be acceptable for confirmatory procedures.

We consider this deficiency resolved.

Response to 2b:

The petitioner has addressed the nature of the discrepancy satisfactorily and shown that it was not due to incomplete hydrolysis of chlorpyrifos-methyl.

We consider this deficiency resolved.

Response to 3a:

Since, in the metabolism studies discussed above, base hydrolysis of grains, meat, milk, poultry and eggs showed that only 5.6 to a maximum of 22.6% of the radioactivity in the commodities was released we are no longer raising any questions with respect to conjugates of metabolites of chlorpyrifos-methyl in these r.a.c.'s.

We consider this deficiency resolved.

Response to 3b:

The petitioner has revised the proposed tolerance levels for the grain byproducts to those recommended in our original review of this petition.

We consider this deficiency resolved.

Response to 4a:

See discussions in 1a, 1b and 3a above.

We consider this deficiency resolved.

Response to 4b:

Based on the metabolism and feeding studies submitted to date it is our judgement that the proposed tolerance levels for meat, milk, poultry and eggs are not acceptable. More appropriate levels would be 0.5 ppm in meat, fat and meat byproducts and poultry, 0.1 ppm in eggs and 1.25 ppm in milkfat (reflecting 0.05 ppm in whole milk). A revised Section F is needed. The petitioner should be so informed.

We do not consider this deficiency resolved.

Other Considerations

The International Tolerance Sheet is attached. Please see our original review regarding the compatibility of the different tolerance levels and regulations.

Conclusions:

1) The nature of the residue in stored grains and animals is adequately understood. The terminal residue of concern will consist of chlorpyrifos-methyl and 3,5,6-trichloro-2-pyridinol.

2) Pending completion of a successful method trial, adequate analytical methods are available for enforcement of tolerances in stored grains, meat, milk, poultry and eggs in terms of

parent compound and the trichloropyridinol. A method trial is being initiated at this time.

3) The presently proposed tolerance levels for meat, milk, poultry and eggs are not acceptable. More appropriate levels would be 0.5 ppm in poultry, meat, fat and meat byproducts, 0.1 ppm in eggs and 1.25 ppm in milkfat (reflecting 0.05 ppm in whole milk). A revised Section F proposing these levels in the subject commodities is needed.

4) The International Tolerance Sheet is attached. Please see our original review regarding the compatibility of the different tolerance levels and regulations.

Recommendations:

We recommend that the proposed tolerances not be established for the reasons given in conclusions 2 and 3 above. Requirements for resolution of these deficiencies are also discussed in the appropriate conclusion above. The petitioner should be informed of these requirements.

Note to Product Manager: When and if these tolerances are established, the regulation will include one metabolite common to both chlorpyrifos and chlorpyrifos-methyl. This metabolite is 3,5,6-trichloro-2-pyridinol. Therefore, a paragraph should be added to Section 180.3(d) stipulating where tolerances are established for both chlorpyrifos and chlorpyrifos-methyl on the same raw agricultural commodity the total amount of such residues shall not exceed the highest established tolerance for either pesticide having this metabolite.

Also, TOX should be made aware of our deferrals to them discussed in the petitioner's response to original deficiencies 1a and 1b above so that a formal reply can be prepared.

R.B.Perfetti:DCR-26341:RCB.11:RM:810:X-7-7737:1-21-83:bje
REVISED:R.B.Perfetti:DCR#26026:RCB11:RM.810:jad:1/24/83

Perfetti

INTERNATIONAL RESIDUE LIMIT STATUS

I.L. 4/17/03

CHEMICAL Chlorpyrifos methyl (O,O-dimethyl O-(3,5,6-trichloro-2-pyridyl) phosphoro thioate) PETITION NO OF2423/ON5271

CCPR NO. 90

Codex Status

☐ No Codex Proposal Step 6 or above

Proposed U. S. Tolerances

Chlorpyrifos methyl and its metabolite 3,5,6-trichloro-2-pyridinol.

Residue (if Step 9): chlorpyrifos – methyl 3/

Residue: See above

Crop(s)	Limit (mg/kg)
milk	0.01 1/
meat of chickens	0.05 1/, 2/
chicken fat	0.05 1/, 2/
chicken byproducts	0.05 1/, 2/
eggs	0.05 1/, 2/
maize	10
rice	0.1
sorghum	10

Cont'd page 2 of 2

Crop(s)	Tol. (ppm)
milk fat	0.1
milk (whole)	0.02
poultry & eggs	0.05
corn grain, barley, oats, rice, sorghum and wheat	6
cattle, goats and sheep meat	0.1
" " " " fat	0.2
" " " " meat byproducts	1.1

(over)

CANADIAN LIMIT

Residue:

Crop	Limit (ppm)
none	

MEXICAN TOLERANCIA

Residue:

Crop	Tolerancia (ppm)
none	

1/ at or about limit of determination

Notes: 2/ Step 5, but recommended omission of step 6&7 (in effect should be treated as if a step 6).

3/ Determination needs to be made residue-wise and Tox-wise whether U.S. expression can be made compatible. Consideration should also be given to harmonizing commodity expression where possible.

INTERNATIONAL RESIDUE LIMIT TATUS

CHEMICAL Chlorpyrifos-methyl PETITION NO OF2+25/0A5527

CCPR NO. 90

Codex Status

[ ] No Codex Proposal
Step 6 or above

Proposed U. S. Tolerances

Residue (if Step 9): _____

Chlorpyrifos-methyl

Residue: _____

parent + 3,5,6-trichloro-2-pyridin

Crop(s)	Limit (mg/kg)
Contd from p. 1 of 1	
wheat	10
Carcase meat of cattle	0.05 4,3
cattle fat	0.05 4,3
cattle meat byproducts	0.05 4,3
wheat bran	20
flour	2
white bread	0.5
whole meal bread	2

Crop(s)	Tol. (ppm)

CANADIAN LIMIT

Residue: _____

_____

Crop	Limit (ppm)

MEXICAN TOLERANCIA

Residue: _____

_____

Crop	Tolerancia (ppm)

Notes:

13544

006897

Chemical:	**Chlorpyrifos-methyl (ANSI)**
PC Code:	**059102**
HED File Code	**11000 Chemistry Reviews**
Memo Date:	**01/25/1983**
File ID:	**00000000**
Accession Number:	**412-01-0166**

HED Records Reference Center
05/23/2001